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Binding Properties and Photoperiodic Influence of Follicle-Stimulating Hormone Receptors in the Subtropical Wild Quail

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ABSTRACT—The knowledge on gonadotropin receptors in the wild avian species inhabiting in the subtropical zone is scanty. Basic properties and photoperiodism of follicle-stimulating hormone (FSH) binding to the testis or ovary of adult rain quails found abundantly in fields of northern India were studied. The binding of radioiodinated rat FSH to the particulate fraction of testicular homogenates of rain quails was competitively inhibited by mammalian FSHs but not by prolactin (PRL). Mammalian luteinizing hormones (LHs) showed some competition only at high concentrations. The Scatchard plot analysis of the binding of FSH showed a straight line, suggesting the presence of a single class of FSH-binding sites. The mean equilibrium constant of dissociation (Kd) of the specific binding and the number of binding sites were 1.16 (0.88–1.71, 95% confidence interval) nM and 3.06 (2.52–4.11) fmol/mg tissue. Adult males transferred from short-day (SD) to long-day (LD) photoperiods showed marked increases in testicular weight and total FSH binding per testis. FSH binding per unit weight tended to increase at the initial phase of photostimulation. In contrast to the male, photostimulated females showed no significant effect of LD exposure on the total FSH binding per ovary. The changes in ovarian weight after LD exposure were much smaller than those in testicular weight and the changes from the control (SD) value were statistically not significant. These results suggest that 1) specific FSH receptors are present in the gonad of the subtropical wild quail, and their binding properties were basically the same to those of FSH receptors previously reported in several temperate birds including domestic quails, 2) photoperiod is an effective environmental factor in the regulation of FSH binding to the testis but the ovarian FSH binding is not altered by LD photoperiods in this quail, and 3) photoperiodic effects on testicular FSH binding are accompanied by pronounced changes in the testicular weight.

INTRODUCTION

The activity of gonadal function in most species of wild birds shows a seasonal variation, and is regulated by external environmental and internal hormonal factors. Gonadotropins are essential hormones for the gonadal function, and the initial event of gonadotropin action is the binding to its specific membrane receptors in the gonads [1–3]. Photoperiod is an important environmental factor in the regulation of annual changes in the gonadal activity in most temperate birds, and the gonadal growth takes place under long-day (LD) photoperiods [4]. In the subtropical zone, the reproductive seasons are relatively scattered throughout the year, and several environmental factors may be at work [5]. According to Chandola and Thapliyal [6], the gonadal growth in the spotted munia (*Lonchura punctulata*) began with the first monsoon showers and the regression coincided with the end of the monsoon. They [6] further demonstrated that exposing the munia to constant photoperiods had no influence on the reproductive cycle and that short-day (SD) photoperiods caused the gonadal growth. In contrast, the situation in the

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Baya weaver finch (*Ploceus philippinus*) approximated much more to temperate birds. Pavgi [7] indicated that the seasonal variation of gonadal activity in this finch was photoperiodically regulated. Thus, information on photoperiodic influences on gonads is still confusing in subtropical birds. Therefore, study of the effects of photoperiod on the gonadal activity and gonadotropin receptors certainly provides useful information on endocrine control mechanisms of the changes in gonadal function in wild avian species inhabiting in the subtropical zone.

Previous studies have extensively demonstrated FSH receptors in the testis of several species of temperate birds, such as white-crowned sparrow [1], domestic fowl [8, 9], turkey [9] and domestic quail, i.e., Japanese quail [3, 10, 11]. Similarly, the knowledge of ovarian FSH receptors has been accumulated in the temperate birds, such as domestic hen [12, 13] and turkey [9]. Photoperiodic responsiveness of FSH receptors has been reported in two male avian species (white-crowned sparrow and Japanese quail) among the temperate species [1, 10]. However, to the best of our knowledge the study on FSH receptors in the subtropical wild species has not yet been reported.

In the present study, we used the wild quail population, rain quail, inhabiting in the subtropical zone in northern India. First purpose of this study is to characterize the basic properties of FSH binding to the gonad. The other purpose is to determine the photoperiodic influence on gonadal FSH binding in this subtropical birds. We will present evidences suggesting the presence of specific FSH receptors and the sex difference in their photoperiodic response.

MATERIALS AND METHODS

Animals

Adult rain quails, *Coturnix coromandelica,* found abundantly in fields of northern India were used in the present study. All of the subtropical wild quails were obtained from local suppliers in June, 1987 and kept in wiremesh cages (50×30×23 cm) in the aviary under SD photoperiods (6-hr light, 18-hr dark) at natural ambient temperature (maximum monthly average, 35°C in May; minimum monthly average, 14°C in December) with supply of commercial food and water *ad libitum.* Birds were divided into five groups of five birds each. The first group was transferred to LD photoperiods (18-hr light, 6-hr dark) on 30th August. The second, third and fourth groups were transferred to LD on 15ths September, October and November, respectively. The last group maintained on SD throughout served as controls. The day of transfer to LD photoperiods was designated as day 0. Birds in all groups were simultaneously sacrificed by decapitation on 25th November, 1987.

Receptor preparations

Immediately after blood collection, the testes and ovary were removed and weighed on a torsion balance to the nearest 0.5 mg. They were snap-frozen on dry ice-ethanol and stored at −80°C or on dry ice (a few days during sample-transportation from India to Japan) until the binding assay for FSH was performed in January, 1988. The frozen samples were rapidly thawed and homogenized in cold Tris-HCl buffer (0.04 M; pH 7.4) containing $MgSO_4$ (5 mM) and 0.1% BSA. The homogenates were centrifuged at 11,000×*g* for 20 min at 4°C. The resulting pellets were resuspended in cold buffer and adjusted to contain 4 mg equivalent wet tissue/100 μl as the receptor preparation. A part of receptor preparations in the first group was used to characterize basic properties of FSH receptors.

Hormone preparations

Highly purified rat FSH (NIDDK-rFSH-I-6) was radioiodinated for the assay of FSH receptors. Unlabeled NIDDK-rFSH-I-6, NIDDK-ovine (o)FSH-17, NIH-FSH-P-2, NIDDK-rLH-I-5, NIDDK-oLH-25 and NIDDK-rPRL-I-4 were used as competitors for competition-binding experiments. Unlabeled NIH-FSH-P-2 was used to correct for nonspecific binding throughout the assay of FSH receptors.

Binding assay

For the assay of FSH receptors NIDDK-rFSH-I-6 was radioiodinated with ^{131}I ($Na^{131}I$, Radio-

chemical Centre, Amersham, United Kingdom) in the presence of lactoperoxidase and hydrogen peroxide using the method described previously [10, 11]. The specific activity of labeled FSH was 52 μ Ci/μg calculated by our previous method [14]. Due to the small amount of membranes available in the gonads of rain quails, most of the binding experiments were performed using a micro-radioreceptor assay (RRA) described previously [14–16]. The volume of the reaction mixture in the micro-RRA (90 μl) was approximately a half of the standard assay system (200 μl; [17]). The precision index (λ) was less than 0.17 in the micro-RRA and 0.10 in the standard RRA. For the FSH-binding assay, receptor preparation (4 mgeq original tissue/100 μl in the standard RRA; 2 mgeq tissue/50 μl in the micro-RRA) and [^{131}I]iodo-rFSH (0.98 ng/50 μl in the standard RRA; 0.39 ng/20 μl in the micro-RRA) were incubated at 37°C for 3 hr with or without unlabeled NIH-FSH-P-2 (20 μg/50 μl in the standard RRA; 8 μg/20 μl in the micro-RRA). In the saturation binding experiments, different amounts of [^{131}I]iodo-rFSH (0.15–4.9 ng; 20 μl) and receptor preparations (2 mgeq original tissue; 50 μl) were incubated with or without an excess amount of cold NIH-FSH-P-2 (2.5–80 μg; 20 μl). At the end of incubation, 1 ml cold Tris-HCl buffer (0.04 M; pH 7.4) containing 5 mM $MgSO_4$ and 0.1% BSA was added to each tube, and the tubes were centrifuged at 11,000×g for 3 min at 4°C. The pellets were washed twice with cold buffer, and the radioactivity of resultant pellets was counted in an autowell γ-counter. Before the experiments, all reaction tubes had been coated with BSA. Scatchard plots were constructed from the data obtained from the saturation binding experiment. The equilibrium constant of dissociation (Kd) and the number of binding sites were determined from the Scatchard plots. A straight line was fitted to the plots by the method of least squares.

Statistical analysis

To compare the patterns of changes in gonadal weight and binding capacity after transfer from SD to LD between the male and female, rates of changes in these parameters from each control value were employed to normalize indices. The gonadal weight and binding capacity after photostimulation were expressed as the ratio to the mean value of each parameter of the control. Results were expressed as the mean±SEM and were analyzed for significance of difference by Bartlett test, followed by Duncan's multiple range test [18]. Statistics for linearity, precision and 95% confidence interval were computed according to the method of Bliss [19].

RESULTS

Binding properties of FSH receptors in the subtropical wild quail

Figure 1 shows the effect of incubation time on FSH binding to the testis of the subtropical wild quail. When 0.98 ng of [^{131}I]iodo-rFSH and the receptor preparation derived from 4 mgeq wet tissue were incubated by the standard RRA system (200 μl per assay tube), specific binding of [^{131}I] iodo-rFSH increased rapidly during the first 1 hr of incubation at 37°C and tended to reach a plateau after 3 hr.

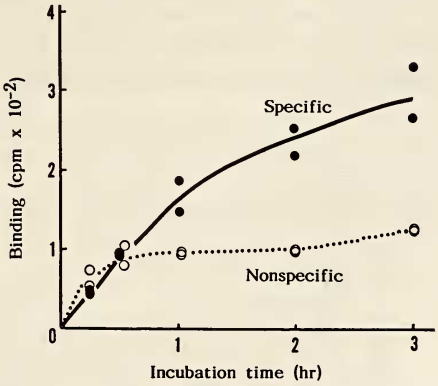

FIG. 1. Binding of [^{131}I]iodo-rFSH to the particulate fraction of testicular homogenates of the rain quail as a function of incubation time. Incubation of standard RRA at 37°C. Solid and open circles represent specific and nonspecific bindings of duplicate determinations.

In order to examine the ligand specificity of FSH binding to the testis, competition experiments were performed by the micro-RRA system (0.39 ng labeled NIDDK-rFSH-I-6 and 2 mg testicular

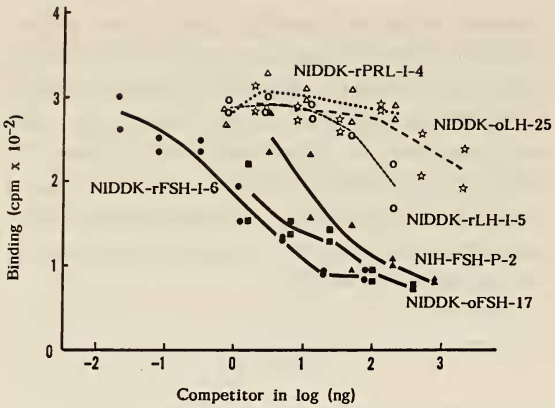

FIG. 2. Competition of specific binding of [^{131}I]iodo-rFSH to the particulate fraction of testicular homogenates of the rain quail by various gonadotropin preparations (NIDDK-rFSH-I-6, NIDDK-oFSH-17, NIH-FSH-P-2, NIDDK-rLH-I-5 and NIDDK-oLH-25) and NIDDK-rPRL-I-4. Incubation of micro-RRA for 3 hr at 37°C.

tissue; 90 μl per assay tube) using NIDDK-rFSH-I-6, NIDDK-oFSH-17, NIH-FSH-P-2, NIDDK-rLH-I-5, NIDDK-oLH-25, and NIDDK-rPRL-I-4 as competitors. When three kinds of FSH preparations were used as the competitors, the binding of [^{131}I]iodo-rFSH was inhibited as a function of the concentration of the competitor (Fig. 2). In contrast, NIDDK-rPRL-I-4 failed to inhibit FSH binding, and NIDDK-oLH-25 tended to slightly inhibit only at high concentrations (>2.0 μg). Although NIDDK-rLH-I-5 showed some competition at high concentrations, the inhibitory potency was clearly less than any FSH preparations (e.g., rLH-I-5 *vs.* FSH-P-2, *ca.* 1:32). The FSH contamination of NIDDK-rLH-I-5 was less than 0.04×NIH-FSH-S1 (data from NIDDK, U.S.A.). The biological potency of NIH-FSH-P-2 was 0.69×NIH-FSH-S1 (data from NIDDK). Therefore, it may be considered that the FSH contamination of NIDDK-rLH-I-5 is less than 0.058×NIH-FSH-P-2 (e.g., rLH-I-5 *vs.* FSH-P-2, *ca.* 1:>17). The inhibitory potency of NIDDK-rLH-I-5 might be due to the contamination of FSH in this preparation.

The effect of receptor concentration on the binding level was examined in this experiment. The receptor preparation of various concentrations ranging from 0.5 to 8 mgeq wet tissue and 0.98 ng of [^{131}I]iodo-rFSH were incubated by the standard RRA system (200 μl per tube). Specific binding of labeled FSH was increased as a function of receptor concentration (Fig. 3). To examine the saturability of FSH binding to the testis, saturation-binding experiments were conducted by the micro-RRA system (2 mg testicular tissue and 0.15–4.9 ng labeled FSH; 90 μl per tube). As shown in Figure 4, specific binding of [^{131}I]iodo-

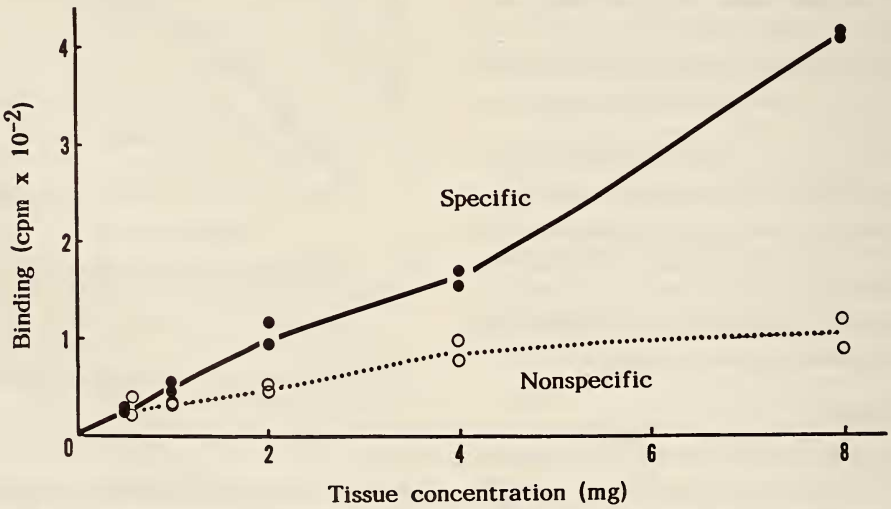

FIG. 3. Binding of [^{131}I]iodo-rFSH to the particulate fraction of testicular homogenates of the rain quail as a function of receptor tissue concentration. Incubation of standard RRA for 3 hr at 37°C. Solid and open circles represent specific and nonspecific bindings.

rFSH tended to saturate with respect to the concentration of labeled FSH. In contrast, nonspecific binding increased linearly. Scatchard plots were constructed from the data of this experiment (Fig. 4). A straight line could be fitted to the plots ($P<0.05$). However, there was a rather large variation in Scatchard plots (Fig. 4). The mean apparent dissociation constant (KD) and the number of FSH-binding sites calculated from the slope of the fitted straight line were 1.16 nM (0.88–1.71 nM, 95% confidence interval) and 3.06 (2.52–4.11) fmol/mg tissue, respectively.

Photoperiodisms of testicular and ovarian FSH bindings in the subtropical wild quail

As shown in Figure 5, *upper panel,* transfer from SD to LD induced a marked increase in testicular weight, though the testis in the SD (control) group remained small ($P<0.05$, SD *vs.* LD-day 71; $P<0.01$, SD *vs.* LD-day 87). The specific binding of [^{131}I]iodo-rFSH per unit testicular weight (density of FSH binding) tended to increase up to 41 days of photostimulation (SD *vs.* LD-day 41, *ca.* 1 : 1.9), but the alteration was not significant (Fig. 5, *middle panel*). In contrast, FSH binding per testis (total FSH binding) markedly increased during photostimulation ($P<0.05$, SD *vs.* LD-day 71; $P<0.01$, SD *vs.* LD-day 87; Fig. 5, *lower panel*).

On the other hand, the changes in ovarian weight after LD exposure were much smaller than those in testicular weight and the changes from the control SD value were statistically not significant (Fig. 5, *upper panel*). As shown in Figure 5, *middle panel,* the density of FSH binding was almost constant during photostimulation. Unlike in the testis, there was no significant effect of LD

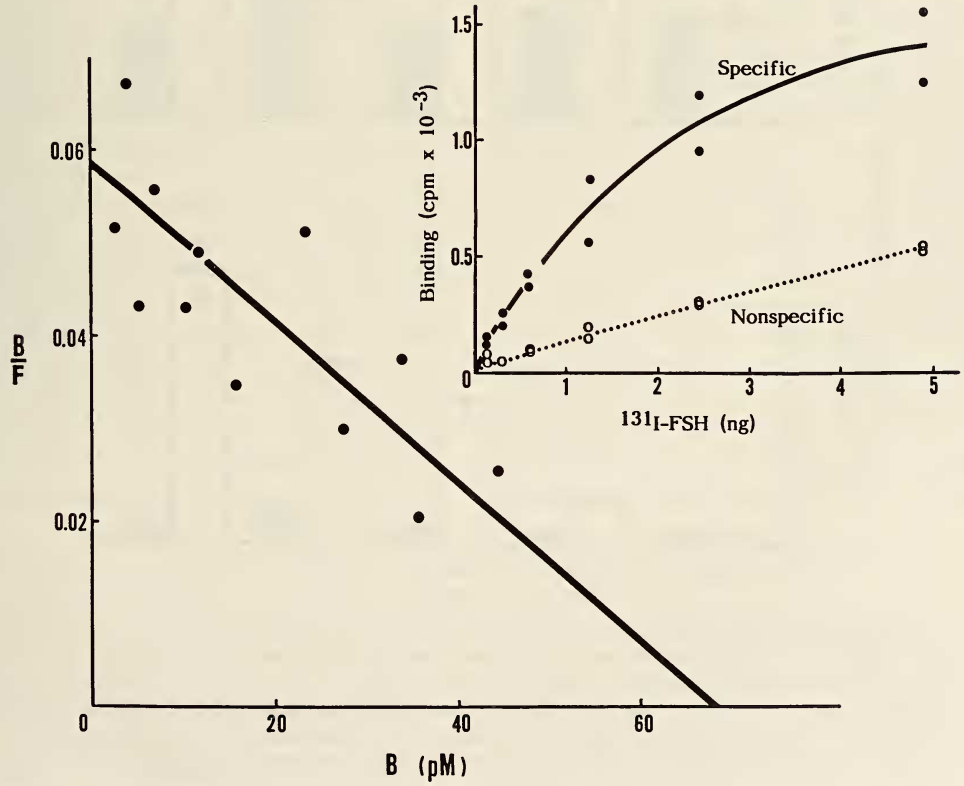

FIG. 4. Scatchard plots of the binding of [^{131}I]iodo-rFSH to the particulate fraction of testicular homogenates of the rain quail. B, Concentration of bound hormone at apparent equilibrium; F, concentration of free hormone at apparent equilibrium. Inset, specific (solid circle) and nonspecific (open circle) bindings in the saturation binding experiment. Incubation of micro-RRA for 3 hr at 37°C.

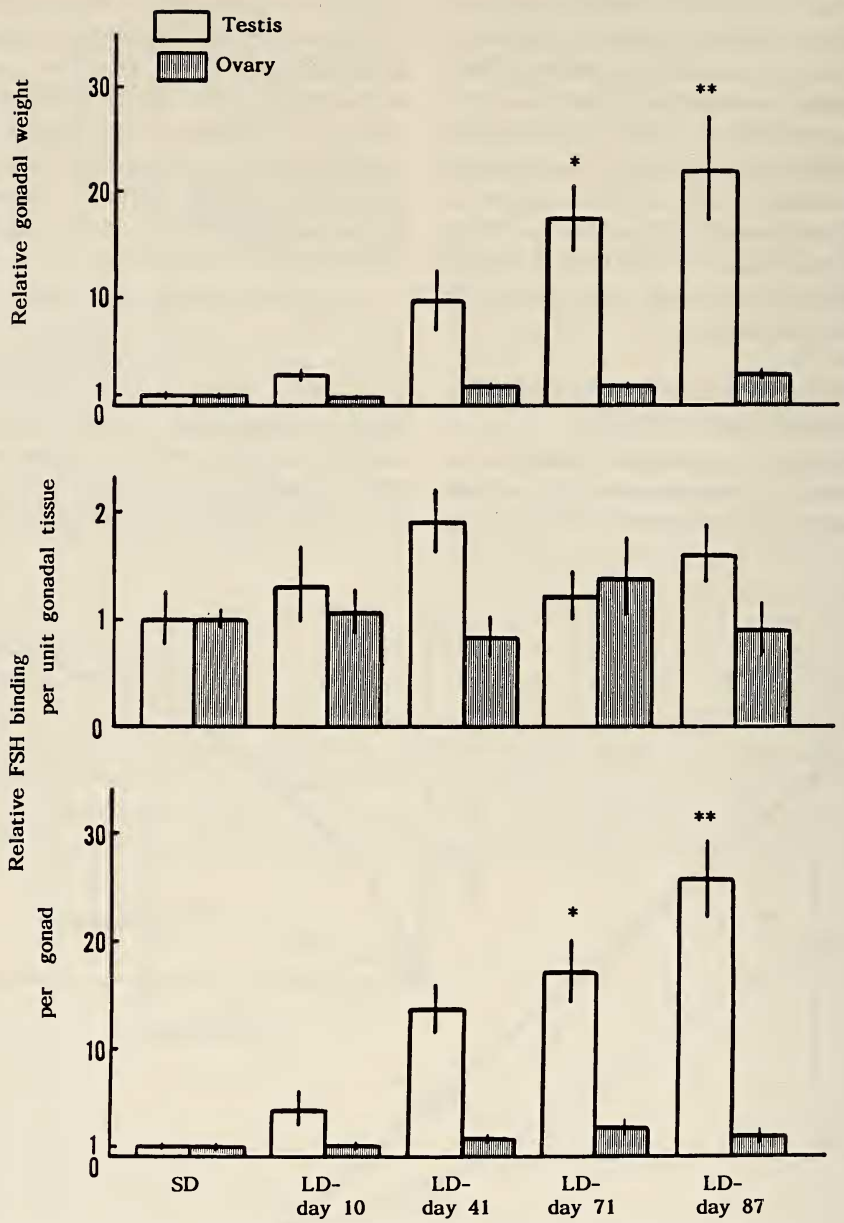

Fig. 5. Changes in the gonadal weight in rain quails after transfer to LD or maintained in SD (control) (upper panel), specific binding of [^{131}I]iodo-rFSH per unit gonadal tissue (middle panel), and total specific binding of [^{131}I]iodo-rFSH per gonad (lower panel). Incubation of micro-RRA for 3 hr at 37°C. The relative gonadal weight and binding capacity after photostimulation were expressed as the ratio to the mean value of each parameter of the SD control. Each solid (ovary) or open (testis) column and vertical line represent the mean ± SEM (n=5 in each group). Significant difference from the SD control by Duncan's multiple range test: $^{*}P<0.05$; $^{**}P<0.01$.

exposure on the total FSH binding in the ovary (Fig. 5, *lower panel*).

DISCUSSION

Previous studies using rat FSH as a ligand have demonstrated specific FSH receptors in the testis of the domestic quail strain, Japanese quail, inhabiting in the temperate zone [3, 10, 11]. In the present study, we detected the specific binding of rat FSH to the testis and ovary of the subtropical wild quail population, rain quail. Most of the physicochemical properties of FSH binding to the testis of this species, such as hormone specificity, time course to equilibrium, relation between receptor concentration and binding, and affinity (Kd) were basically the same to those of the specific FSH receptors reported in the Japanese quail [3, 10, 11] and other temperate birds [1, 8, 9].

Competition-binding experiments suggested that FSH receptors in the testis of the rain quail specifically recognize mammalian FSHs but not LHs or rPRL. Scatchard plots yielded a linear regression line, which suggests the presence of a single class of binding sites for FSH. As shown in Table 1, the Kd was 1.16 (0.88–1.71, 95% confidence interval) nM, which was not much different from the values of specific FSH receptors previously reported using the same radioligand in the Japanese quail (Kd=4.1 nM by Ishii and Adachi [3]; 0.52 nM by Tsutsui and Ishii [10]). In addition, the present Kd value was very close to those of other avian species using the same radioligand (in the white-crowned sparrow: Kd=0.78 nM, Ishii and Farner [1]; in the domestic fowl: Kd=1.5 nM, Ishii and Adachi [3], Table 1).

The present study further demonstrated the clear sex difference in photoperiodic responsiveness of FSH binding to the gonads in the subtropical wild quail. The results of photostimulation experiments revealed that the ovarian FSH binding was stable but that FSH-binding capacity in the testis changed markedly. The male rain quails responded to the artificial LD condition by showing not only marked increases in the testicular weight but also in the total FSH-binding capacity in the testis. The density of FSH binding tended to increase at the initial phase of photostimulation. Tsutsui and Ishii [8] also reported that in the photostimulated immature Japanese quail the total FSH binding continued to increase throughout the period of testicular growth, while the increase in density of FSH binding was observed only at the initial phase of testicular growth. Furthermore, these results in two quail species are in agreement with the findings in the domestic fowl [8]. Thus, it is generally stated that the testicular development is accompanied by changes in FSH receptors in birds regardless of the species and inhabiting zone.

In addition to FSH-binding capacity, the present study showed that in the rain quail photoperiodic influence on the weight of gonads, a parameter of gonadal activity, is much larger in the male than in the female. Such a sex difference in photoperiod-related changes may be due, at least partly, to the sex difference in the photoperiodic responsiveness of FSH-binding capacity. There may be another possibility that the responsiveness of circulating gonadotropin levels was different between the sexes. However, this possibility may be low, because in male Indian weaver birds, another subtropical wild species, the FSH and LH levels

TABLE 1. Comparison of affinity (Kd) and capacity of FSH binding to the testis in the subtropical and temperate birds

Region	Source of receptor	Source of FSH	Kd (nM)	Capacity (fmol/mg tissue)	Reference
Subtropical zone	Wild quail	Rat	1.16	3.06	Present study
Temperate zone	Domestic quail	Rat	4.1	6.4	[3]
	Domestic quail	Rat	0.52	2.3	[10]
	Domestic fowl	Rat	1.5	11.2	[3]
	Wild sparrow	Rat	0.78	7.0	[1]

after photostimulation were almost the same to those in the female [Kawashima *et al.*, unpublished observation]. Sex difference in the photoperiodism of gonadal weight was also reported in the temperate bird, white-crowned sparrow [20]. On the other hand, some studies using another wild avian species suggested the difficulty in inducing the ovarian development due to the failure in the yolk accumulation under the artificial breeding condition. We cannot exclude the possibility that the breeding condition established in the present study was not suitable for the ovarian development in rain quails.

It is well known that in the Japanese quail [10, 11] as well as several species of mammals, i.e., prepubertal rat [14, 21, 22], mouse [17] and photostimulated hamster [16], the changes in FSH binding during testicular development are due to changes in the number of FSH-binding sites. It is possible that the increase in total FSH-binding capacity of the photostimulated rain quail reflects the increase in the number of binding sites in the testis. It has previously been confirmed in the rat, mouse and Japanese quail that FSH-binding sites in the testis are mainly localized in Sertoli cells, as ascertained by autoradiography and binding studies [8, 23–25]. The increase in total FSH binding in the rain quail testis after transfer from SD to LD may be explained either by the increase in FSH binding per Sertoli cell or the increase in the number of Sertoli cells without changes in the density of binding per cell. There is an evidence indicating that the number of Sertoli cells per testis is stable throughout the season in the adult hamster [26] and ram [27] and after puberty in the rat [28]. Therefore, the possibility that the Sertoli cell number is virtually constant and that FSH binding per cell increases may be more feasible in adult rain quails during the LD-induced testicular development.

In contrast to the testis, we could not detect any clear-cut influence of LD exposure on the capacity of ovarian FSH binding in adult rain quails. In this conjunction, a decrease in FSH-binding capacity during the course of follicular growth has been demonstrated in the theca layer or granulosa cells in the temperate domestic hen [12, 13, 29]. In the present study, we measured the binding level using homogenates of the whole ovary. Detailed experiments using the separate follicular layer or dispersed follicular cells are needed to establish whether ovarian FSH receptors are generally stable under photoperiodic manipulations in the subtropical wild quail.

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